[0019] FIG. **7**. Antibody treated Vil1-Grem1 animal phenotype after 6 weeks of treatment with anti-Grem1 antibody. Antibody treatment normalises small intestinal phenotype and prevents profound polyposis development. Antibody treatment prevents villus ectopic crypt formation and normalises cell fate determination with appropriate restriction of Ki67, Sox9, EphB2 and lysozyme staining to the base of the intestinal crypt, and resolution of normal CK20 staining in the differentiating cells of the small intestinal villus.

[0020] FIG. **8**. Kaplan-Meier plots of survival of Grem1 initiated polyposis mouse strains following long term anti-Grem1 antibody treatment.

[0021] FIG. **9**. Stromal Grem1 in Apc-driven tumourigenesis. A. Grem1 ISH on wildtype and Villin-CreERT2; Apc fl/fl mice showing profound upregulation of stromal Grem1 in response to acute epithelial Apc loss. B. Transgenic deletion of stromal Grem1 in ApcMin mice reduces mutant Apc tumour burden at 285 days. C. Prolonged treatment with anti-Grem1 antibody (30mg/kg/week) reduces ApcMin tumour burden and prolongs animal lifespan.

[0022] FIG. **10**. Grem1 expression is elevated in primary stromal cultures from myeloma patients. RNA was extracted from bone marrow trephine derived stromal cell cultures from age and gender matched healthy donors and myeloma patients and the expression on Grem1 was analysed by real-time PCR. The expression of Grem1 was significantly higher in the myeloma patient cohort comparative to healthy donors. Data presented as mRNA expression normalised to ActB. (mean±SD, **P<0.001, T-test, Normal; n=17 and MM;

[0023] n=15).

[0024] FIG. **11**. Grem1 expression was (A) analysed in the compact bone of healthy C57B16/KaLwRij.Hsd mice and mice injected with 5TGM1.Bmx1 MM PCs with disease detectable via BLI. (mean±SD, P=0.1120, T-test, Normal; n=11 and Tumour-bearing; n=9). (B) Grem1 expression in the BM stroma isolated from the hind limbs of tumour-bearing mice was correlated with the tumour burden in the respective limbs, as detected by BLI (Pearson Correlation; p<0.05, R2=0.414).

[0025] FIG. **12**. Grem1 expression was analysed in the murine, bone-marrow derived stromal OP9 cell lines following 24, 48 and 72 hr co-culture with (A) 5TGM1.Bmx1 cells, co-cultured in the upper 3 μm transwell (B) 5TGM1. parental cells, plated directly onto the adherent stromal cells. For contact culture, OP9.GFP+ cells were sorted by FACS from the 5TGM1.parental MM PCs for analysis of Grem1 expression via real-time PCR. A significant increase in Grem1 expression was observed in the OP9 stromal cells following 72 hrs of co-culture. Data presented as mRNA expression relative to ActB and normalised to media only control. (mean±SEM of 3 replicate experiments, *P<0.05, t-test).

[0026] FIG. **13**. Gremlin1 expression was analysed in normal human stroma co-cultured with various human MM cell lines for 72 hrs. MM cells were washed from stroma prior to collection for analysis of Grem1 expression. Significant increase in Gremlin1 expression in KMS-11 (p=0.0159) and U266 (p=0.0343) co-culture conditions (ANOVA). Data presented as replicates from co-culture with three separate normal stroma donors, normalised to media only control.

[0027] FIG. **14**. Grem1 transgene expression in OP9-stromal cells was confirmed by (A) RT-PCR and (B) Western blot. 5TGM1 MM PCs in (C) cell contact (D) transwell co-cultures with OP9-stromal cells modified to overexpress Grem1 displayed increased rates of proliferation compared to co-culture with OP9 vector-only controls, as measured by relative luciferase activity. (Mean±SEM of 3 replicate experiments, ** P<0.01, ***P<0.001, t-test).

[0028] FIG. **15**. (A) C**57**B16/KaLwRij mice injected with 5TGM1.Bmx1 MM PCs and subsequently treated with a Grem1-neutralising antibody displayed a significant reduction in overall tumour burden compared to mice treated with an IgG control after 4 weeks as shown by BLI. Mean±SEM, n=13 mice per group ****P<0.0001, one-way ANOVA. (B) SPEP was performed on mice bled at week 4 post-tumour cell injection. M-spike intensity relative to serum albumin was used a measure of disease burden. Mice treated with the anti-Grem1 antibody had significantly lower M-spike intensity compared to mice receiving the IgG control treatment. Mean±SD, **P<0.01, t-test. (C) Representative BLI ventral scan images for IgG control and Grem1-neutralising antibody treated mice.

[0029] FIG. **16**. **5**TGM1 MM cells were co-cultured in 3 μm transwells in the presence of OP9-Grem1 overexpressing cells or vector-only controls for 72 hrs. Lysate from the 5TGM1 cells was analysed by Western blot for phosphorylation of Smads 1/5/9. 5TGM1 cells display a reduction in phosphorylation of Smads 1/5/9 when cultured in the presence of Grem1-overexpressing BM stromal cells, compared to vector-only controls. Hsp90 was used as a loading control. Image representative of two replicate experiments.

[0030] FIG. **17**. The BM stromal cell line, OP9 was assessed for expression of Grem1 following treatment with 20 ng/ml of recombinant IL6 for 72hrs. A significant increase in Grem1 expression was observed in the OP9 cells stimulated with IL6. (Mean±SEM of 3 replicate experiments, ** P<0.01).

[0031] FIG. **18**. (A) C**57**B16/KaLwRij mice that received treatment of Grem1-neutralising antibody (Ab7326) prior to inoculation with 5TGM1.Bmx1 MM PCs displayed a significant reduction in overall tumour burden at 4 weeks post-cell inoculation, compared to mice treated with that received an IgG control (Ab101.4), as shown by BLI. Mean±SEM, n=7-8 mice per group ****P<0.0001, one-way ANOVA. (B) SPEP was performed on mice bled at week 4 post-tumour cell injection. M-spike intensity relative to serum albumin was used a measure of disease burden. Mice treated with the anti-Grem1 antibody had significantly lower M-spike intensity compared to mice receiving the IgG control treatment. Mean±SD, **P<0.0001, t-test. (C) Representative BLI ventral scan images for IgG control and Grem1-neutralising antibody treated mice 4-weeks post-tumour cell inoculation.

[0032] FIG. **19**. Quantitative RT-PCR analysis of Grem1 mRNA expression. Grem1 gene expression was analysed in (A) human MDA-MB-231-TXSA breast cancer cells and (B) human MF9 mammary fibroblasts following 48hr culture in normoxic and hypoxic conditions (n=1). Expression levels were measured by RT-PCR and normalised to (β-actin. Mean±SD of triplicate wells, *p<0.05, **p<0.005, Student's unpaired t-test.

[0033] FIG. **20**. Human breast cancer cell proliferation in response to stimulation with Grem1. MDA-MB-231-TXSA cells were stimulated with various concentrations of rhGrem1 and cultured under normoxic (left-hand bar of each pair) and hypoxic conditions (right-hand bar of each pair)